

# Fluctuation assays

RB R Blake Billmyre

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 An abbreviated version of this protocol was published in eLIFE in Sep 2017

Natural mismatch repair mutations mediate phenotypic diversity and drug resistance in *Cryptococcus deuterogattii*

DOI: 10.7554/eLife.28802

## Detailed protocol

Hi Xiaoli,

Thanks for reading our paper and for the questions.

Our choice of drug concentration was based on standard concentrations used in the field for *Cryptococcus*. The 5-FOA fluctuation assay was previously used (<https://doi.org/10.1371/journal.ppat.1002936>) in a fluctuation assay for *Cryptococcus* as cited in the paper. The combination of FK506 and Rapamycin was using the standard concentrations we used in the lab for each individually, but in combination. If you are trying to do a fluctuation assay using a different drug or a different organism, you should determine empirically what concentration is sufficient to inhibit growth while still allowing the possibility of mutations that confer resistance. This should ideally be done in a separate pilot experiment prior to a full-scale fluctuation assay. We did this type of pilot for the combination of FK506/Rapamycin to be sure that we could recover resistant isolates.

You are correct that FALCOR is no longer available online- I was similarly displeased to make that discovery in the past couple years. I unfortunately don't have a back-up to access FALCOR itself, but have successfully used <http://shinyflan.its.manchester.ac.uk/> as a replacement since for fluctuation assays.

Thanks,

Blake Billmyre

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Billmyre, R. (2021). Fluctuation assays. Bio-protocol Preprint. [bio-protocol.org/prep821](https://bio-protocol.org/prep821).
2. Billmyre, R. B., Clancey, S. A. and Heitman, J.(2017). Natural mismatch repair mutations mediate phenotypic diversity and drug resistance in *Cryptococcus deuterogattii*. eLIFE. DOI: [10.7554/eLife.28802](https://doi.org/10.7554/eLife.28802)

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